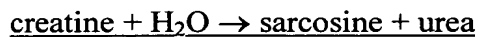


## CLAIM AMENDMENTS

1.-32. (canceled)

33. (thrice amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:



K<sub>m</sub> values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM

Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

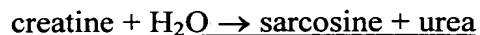
Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point of 4.5.

34. (canceled)

35. (thrice amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:



K<sub>m</sub> values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 4.5±1.0 mM.

Optimum temperature: about 40-50° C (at pH of about 7.5)

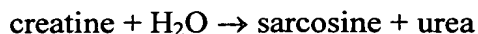
Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

36. (thrice amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:



K<sub>m</sub> values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 6.5±1.0 mM.

Optimum temperature: about 40-50° C (at pH of about 7.5)

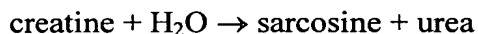
Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

37. (thrice amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:



K<sub>m</sub> values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 9.0±1.0 mM.

Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

38. (once amended) A method for producing the creatine amidinohydrolase of claim 33, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

39. (once amended) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 33, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

40. (once amended) A method for determining creatine in a sample, which comprises measuring absorbance of a pigment produced by the reaction of the reagent of claim 39 with the sample.

41. (once amended) A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim 33, a

sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

42. (once amended) A method for determining creatinine in a sample, which comprises measuring -absorbance of a pigment produced by the reaction of the reagent of claim 41 with the sample.

43. (once amended) A method for producing the creatine amidinohydrolase of claim 35, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

44. (once amended) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 35, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

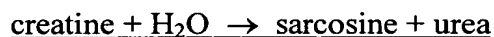
45. (once amended) A method for determining creatine in a sample, which comprises measuring -absorbance of a pigment produced by the reaction of the reagent of claim 44 with the sample.

46. (once amended) A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim 35, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

47. (once amended) A method for determining creatinine in a sample, which comprises measuring -absorbance of a pigment produced by the reaction of the reagent of claim 46 with the sample.

48. (twice amended) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:



Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

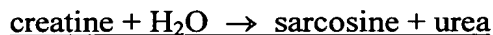
K<sub>m</sub> value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase:  
3.5 - 10.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

49. (twice amended) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:



Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

K<sub>m</sub> value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase:  
4.5±1.0 mM

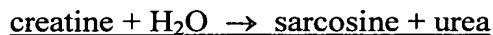
Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

50. (once amended) The creatine amidinohydrolase of claim 49, which is obtained from *Escherichia coli* JM109 (pCRH273M2) (FERM BP-5375).

51. (twice amended) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:



Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

K<sub>m</sub> value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase:  
6.5±1.0 mM

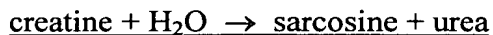
Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

52. (once amended) The creatine amidinohydrolase of claim 51, which is obtained from *Escherichia coli* JM109 (pCRH273M1) (FERM BP-5374).

53. (twice amended) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:



Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

K<sub>m</sub> value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase:  
9.0±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

54. (once amended) The creatine amidinohydrolase of claim 53, which is obtained from *Escherichia coli* JM109 (pCRH273M3) (FERM BP-5376).

55. (not amended) A method for producing the creatine amidinohydrolase of claim 48, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

56. (twice amended) The method of claim 55, wherein said microorganism is selected from the group consisting of *Escherichia coli* JM109 (pCRH273M1) (FERM BP-5374), *Escherichia coli* JM109 (pCRH273M2) (FERM BP-5375), and *Escherichia coli* JM109 (pCRH273M3) (FERM BP-5376).

57. (not amended) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 48, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

58. (not amended) The reagent of claim 57, in which the composition for the detection of hydrogen peroxide comprises an enzyme having a peroxidase activity, a chromophore, and a buffer.

59. (not amended) The reagent of claim 58, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase, and myeloperoxidase.

60. (not amended) The reagent of claim 58, in which the chromophore comprises a hydrogen receptor and a coupler.

61. (not amended) The reagent of claim 60, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

62. (not amended) The reagent of claim 60, in which the coupler is an aniline derivative or a phenol derivative.

63. (once amended) A method for determining creatine in a sample, which comprises measuring absorbance of the pigment produced by the reaction of the reagent of claim 57 with the sample.

64. (once amended) A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim 48, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

65. (not amended) The reagent of claim 64, in which the composition for the detection of hydrogen peroxide comprises an enzyme having a peroxidase activity, a chromophore, and a buffer.

66. (not amended) The reagent of claim 65, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase, and myeloperoxidase.

67. (not amended) The reagent of claim 65, in which the chromophore comprises a hydrogen receptor and a coupler.

68. (not amended) The reagent of claim 67, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

69. (not amended) The reagent of claim 67, in which the coupler is an aniline derivative or a phenol derivative.

70. (once amended) A method for determining creatinine in a sample, which comprises measuring absorbance of the pigment produced by the reaction of the reagent of claim 64 with the sample.

Please cancel claims 71-75.